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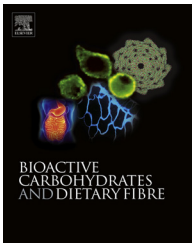

Enzyme resistant carbohydrate based micro-scale materials from sugar beet (*Beta vulgaris* L.) pulp for food and pharmaceutical applications

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ABSTRACT

Bio-based micro-scale materials are increasingly used in functional food and pharmaceutical applications. The present study produced carbohydrate-based micro-scale tubular materials from sugar beet (*Beta vulgaris* L.) pulp (SBP), a by-product of sugar beet processing. The isolated carbohydrates were composed of 84% non-sucrose carbohydrates and small amount of fat (13%), protein (1.22%), and ash (0.9%). These highly pure carbohydrates were used to prepare micro-scale materials. Micro-scale tubular structures had lengths of 22.5 ± 0.9 μm and cavities of 3.9 ± 1.2 μm. The stability of these micro-scale tubular materials was studied by dissolution and digestion studies. The degrees of dissolution under simulated stomach and intestine conditions were 45–56% and 45–58%, respectively. The pancreatic enzymatic digestion with α-amylase and amyloglucosidase was 34.1%. In addition the X-ray diffraction showed the presence of A-type structures with 11.07% crystallinity. Furthermore, the material was thermally stable.

1. Introduction

The sugar beet is the major starting material for sucrose production (crystal sugar) in the United States of America. The Great Lakes region (Michigan and Ohio), the Upper Midwest (Minnesota and North Dakota), the Great Plains (Colorado, Montana, Nebraska, and Wyoming), and the West (California, Idaho, Oregon, and Washington) are the major sugar beet producing regions accounting for more than 55% of the total sugar production in the USA. Among different regions, the Red River valley of Western Minnesota and Eastern North Dakota leads the nation in sugar beet production (FAO, 2012).

Sugar beet pulp, a by-product of sucrose production is a rich source of nutrients. Approximately 29 million metric tons of sugar beet pulp is produced annually by the sugar beet

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sucrose industry— most of them are used as animal feed for its nutritional profile. Major nutrients in sugar beet pulp include: non-sucrose carbohydrates (75–80%), fat (1.4%), protein (10.3%), ash (3.7%), and lignin (5.9%) (Dinand, Chanzy, & Vignon, 1999). Cellulose (22–24%), hemicelluloses (30%), and pectin (25%) are the major components of non-sucrose carbohydrates. The non-sucrose carbohydrate fractions found in sugar beet pulp could be potential materials for food and pharmaceutical applications.

Potential incorporation of non-sucrose carbohydrates in food and pharmaceutical applications is attributable to the unique physical and chemical properties of these carbohydrates. The intra and inter molecular interactions between cellulose and hemicellulose contribute to strength, rigidity, and viscoelastic properties (Sun & Hughes, 1999). In addition, incorporation of the pectin into cellulose and hemicellulose can optimize the physical stability due to intensive interactions between molecules (Sun & Hughes, 1999; Siro & Plackett, 2010). Overall, the physicochemical properties of cellulose, hemicellulose, and pectin suggest that these materials could be used as packaging materials for bioactive compounds (therapeutics and nutrients) in both food and pharmaceutical applications (Elleuch et al., 2011; Zhou, Li, Yan & Xie, 2011).

The specific physicochemical properties possessed by the non-sucrose polysaccharides at macro-scale level can be further improved by reducing their particle size to micro-scale level. At micro-scale level the particles have high surface area as compared to particles at macro-scale. The high surface area of the particles can intensively interact with each other. This phenomenon leads to an enhanced strength, rigidity, and viscoelasticity of the particles (Rashidi & Khosravi-Darani, 2011; Mikkonen & Tenkanen, 2012). Thus, the incorporation of these non-sucrose polysaccharides at reduced particle size in food and pharmaceutical applications can be efficient as compared to the macro-scale materials with the same chemical composition.

Beside the unique physicochemical properties, the micro-scale mixed polymers of non-sucrose polysaccharides isolated from bio-based sources have greater consumer appeal compared to their synthetic counter parts. These polymers are biodegradable; they provide substantial health benefits, reduced blood cholesterol levels, blood sugar, and chronic diseases (diabetes, obesity, and cancer). Owing to the biocompatibility and health benefits, the bio-based polymers are preferred in human applications. Therefore, we focused on isolation, production, and characterization of micro-scale materials from non-sucrose carbohydrates of sugar beet pulp.

2. Materials and methods

2.1. Chemicals

The SBP materials were obtained from the Northern Crop Institute (NCI), North Dakota State University, Fargo, North Dakota. Chemicals including enzymes (α-amylase from *Aspergillus oryzae*; amyloglucosidase from *Aspergillus niger*), and solvents for analytical analyses were purchased from Sigma Aldrich Co. (St. Louis, MO), VWR International (Radnor, PA), EMD Serono Inc. (Rockland, MA), and J.T. Baker Chemicals Co. (Phillipsburg, NJ).

2.2. Sample pre-treatment (Neethirajan, Tsukamoto, Kanahara, & Sugiyama, 2012)

Sugar beet pulp samples were finely ground using a cyclone sample mill (UDY Corporation, Fort Collins, CO). A 50.0 g of this sample was treated with Soxhlet extraction with 500.0 mL of hexane for 2.5 h. The hexane was evaporated using a rotor evaporator at 45 °C (Thermo Scientific Corporation, Brockport, NY). The proteins (and any starch residues) of the defatted SBP (40 g) were removed by steeping in 0.2% sodium hydroxide at 45 °C for 90 min (Neethirajan et al. , 2012). The sample was then centrifuged at 3000 rpm (Beckman Coulter Inc., Brea, CA) for 20 min at room temperature (23 °C). The supernatant was discarded and the precipitate was subjected to continuous washing with 18 MΩ Millipore water (Millipore Corporation, Bedford, MA) until no traces of yellow color were visible to the naked eye. The remaining sample was resuspended in millipore water and pH was adjusted to 7.0 using 50 mM hydrochloric acid. The residue was air dried.

2.3. Isolation of human digestive enzyme resistant carbohydrates

The isolation of human digestive enzyme resistant carbohydrates was adapted from McCleary & Monaghan, 2002. Approximately 1.0 g of the pre-treated material was made wet using 2 mL of 80% ethanol. Volume of 30.0 mL thermostable α-amylase (3000 U mL^{-1}) and 90.0 ml Na-acetate buffer (100 mM, pH 4.5) were added and the mixture was incubated in a water bath at 95 °C for 6 min with continuous stirring on a magnetic stirrer (Henry Troemnor LLC, Thorofare, NJ). The samples were then placed in a 50 °C water bath after which 1 mL of amyloglucosidase (AMG) (350 U mL^{-1}) was added and the mixture was incubated at 50 °C for 30 min at 300 rpm on a mini vortex incubator (VWR international LLC, Radnor, PA). The solution was then centrifuged at 3000 rpm for 15 min at room temperature. The supernatant was removed and the residue was air dried for 24 h.

2.4. Preparation of micro-scale material

The procedure for preparing the micro-scale materials was adapted from previously reported procedure (Neethirajan et al., 2012). One gram of air dried isolated resistant carbohydrate was hydrolyzed with 10.0 mL of 3.16 M sulfuric acid for 5 days at 100 rpm and 40 °C in a mini vortex incubator. At the end of the fifth day, the solution was cooled to room temperature, was neutralized with successive washings with millipore water and was freeze dried using liquid nitrogen.

2.5. Determination of non-sucrose fraction of sugar beet pulp

A 100 mg of defatted and deproteinated SBP sample was added into tubes containing 4.0 mL of pancreatic α-amylase (10 mg/l) and amyloglucosidase (3 U/mL). The contents in the

tube were mixed in a vortex mixer and were incubated at 37 °C for 16 h with a constant stirring at 100 rpm in a mini shaking vortex incubator. The tubes were removed from the incubator exactly after 16 h and 4.0 mL of absolute ethanol was added. The tubes were then centrifuged at 1500 rpm for 10 min. The supernatants were discarded and the pellets were suspended in 2.0 mL 50% ethanol and centrifuged again at 1500 rpm for 10 min. The supernatants were discarded and the pellets were suspended in 50% ethanol and centrifuged at 1500 rpm for 10 min. Once the supernatants were discarded the pellets were air dried.

The pellets were further subjected to determine the non-sucrose carbohydrate fraction; a procedure adapted from Association of Official Analytical Chemists, Total Dietary fiber analysis. The pellets were treated with phosphate buffer (pH 6; 5.0 mL) followed by an addition of α-amylase. The solution was incubated for 30 min at 95 °C. Contents were cooled to room temperature and the pH was adjusted to 7.5, followed by an addition protease incubated at 60 °C for 30 min. Further, 280.0 mL of 95% ethanol was added immediately and was kept for 60 min at room temperature. The residue was collected in pre-weighed crucibles. One portion was tested for protein at N × 6.25 and the other portion was incinerated at 525 °C for 5 h. The total dietary fiber was calculated as follows:

$$\text{TDF} = (\text{Weight of residue} - \text{protein} - \text{ash} - \text{blank})/\text{weight of the pellet}$$

2.6. Chemical dissolution tests (Dimantov, Kesselman, & Shimoni, 2004; Dimantove, Greenberg, Kesslseman, & Shimoni 2004)

The chemical dissolution of micro-scale materials was performed by incubation of 0.1 g materials in solutions simulating stomach and intestine conditions. For simulated stomach conditions 0.1 g of the materials was introduced into vials containing hydrochloric acid (HCl) at pH 1.5 and incubated at 37 °C at 100 rpm. The vials were taken out every 30 min, and were centrifuged at 5000 rpm. Then the supernatants were discarded and the pellets were oven dried overnight at 40 °C. For simulated intestine conditions 0.1 g of the materials were introduced into vials containing phosphate buffer at pH 8.5, and then incubated at 37 °C at 100 rpm. The vials were taken out at every one hour interval, and centrifuged at 5000 rpm. The supernatants were discarded, and the resulting pellets were oven dried overnight at 40 °C. The degree of dissolution was calculated at percentage weight loss.

2.7. Enzymatic digestion tests (Dimantov et al., 2004; Dimantove, Greenberg et al., 2004)

Nearly 0.1 g of the micro-scale sugar beet pulp materials were exposed to enzymatic solution containing pancreatic α-amylase (30 U/ml) and amyloglucosidase (300 U/ml) for 16 h at 37 °C at 100 rpm. These conditions stimulated the intestine environment. The samples were withdrawn at the end of the 16 h, and dried at 40 °C overnight and weighed. The digestion was calculated as percent weight loss of the material.

X- ray diffraction studies were done to study the susceptibility of the materials for pancreatic enzymatic hydrolysis. X-ray powder diffraction analysis was performed using Xpert MPD (phillip, Belgium), operating at 40 mA and 45 kV with Cu. Freeze-dried micro-scale materials were acquired at an angular range of 2θ from 4° to 99° with step-size of 0.05°. Counting time was 2 s per step.

2.8. Thermal analysis

Differential scanning calorimetry (DSC) was performed on a Pyris 6 DSC system (Perkin Elmer, Waltham, MA). The DSC curve was obtained at a temperature range of 0–180 °C at a heating rate of 10 °C/minute under dry nitrogen flow.

2.9. Scanning electron microscopy

Micro-scale materials were observed by a JEOL JSM-6490LV SEM ((JEOL) USA, Peabody, MA) by coating with gold-palladium (Model SCD 030, Balzers, Liechtenstein) at an accelerating voltage of 15 kV. micro-scale materials subjected to chemical dissolution and enzymatic digestion were also observed by SEM in the same manner.

3. Results and discussion

Bio-based materials are increasingly used as packaging and encapsulating materials in nutrients and nutraceutical applications. Most widely used bio-based materials are acid hydrolyzed polysaccharides obtained from corn, wheat, soybean, and potato. Nevertheless, lately polysaccharides from agricultural by-products have gained attention, due their superior nutritional properties, as animal feed. Sugar beet pulp is one of the agricultural by-products, which is rich in non-sucrose carbohydrates compared to other by-products (soybean meal, wheat straw, and distillers' dries grains). However, to the best of our knowledge there has not been any reported data on the use of sugar beet pulp carbohydrates for food and pharmaceutical applications.

In this study, the sugar beet pulp non-sucrose carbohydrates were isolated and acid hydrolyzed to produce micro-scale materials. The sulfuric acid hydrolysis resulted in a unique tubular structure (Fig. 1). The tubular structures were in micro-scale with a length of 22.5 ± 0.9 μm and a diameter of 3.9 ± 1.2 μm. The occurrence of these tubular structures, which we refer to as “stacks of tubules” can be attributed to the penetration of H^+ ions of sulfuric acid in both the surface and the inner amorphous region of the isolated carbohydrates. The penetration of H^+ ions could most likely reduce the particle size, from macro- to micro-scale, due to the cleavage of physical and chemical bonding. Micro-scale materials are currently used in food and pharmaceutical applications to entrap or pack nutrients and nutraceutical. Hence, the unique micro-scale tubular structures resulting from acid hydrolysis could be applied in food and pharmaceuticals to encapsulate bioactive ingredients.

Materials used for micro-encapsulation in human applications should be biocompatible, sustainable, and must be generally recognized as safe (GRAS) for human health (Sozer & Kokini, 2009). The micro-scale materials isolated from sugar beet pulp are bio-based and GRAS. The isolated

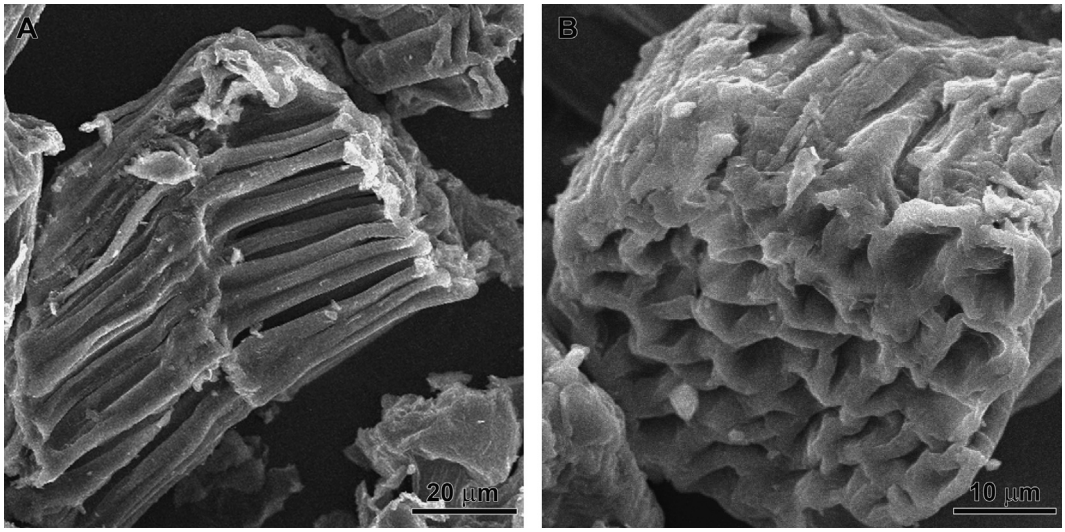

Fig. 1 – Scanning electron microscopy images of sulfuric acid hydrolyzed non-sucrose fraction of sugar beet pulp.

material was composed of non-sucrose carbohydrates (84%), with a small amount of fat (13%), protein (1.22%), and ash (0.9%). Previous studies have shown that the non-sucrose carbohydrates of sugar beet pulp is mainly composed of cellulose, hemicelluloses, and pectin. These carbohydrates provide substantial health benefits such as: reduced cholesterol level, reduced blood glucose level, and prevention of chronic diseases. Thus, the "stacks of tubules" composed of non-sucrose carbohydrates could be potential materials to be incorporated in human applications owing to the health benefits.

3.1. *Dissolution studies*

The non-sucrose carbohydrate rich micro-scale materials produced by acid hydrolysis were further studied for their stability under simulated human physiological conditions. The degree of dissolution of this material under simulated stomach (pH 1.5) and intestine (pH 8.5) conditions are shown in Fig. 2. The degrees of dissolution were significantly different at 95% confidence level; higher dissolution was seen at pH 8.5 (simulated intestine condition). However, it should be noted that in this study the rates of dissolution were high compared to previous studies performed for targeted delivery in the digestive system. The higher degrees of dissolution in the present study can be attributed to the micro-scale material, which has an increased surface area – leading to a higher dissolution. In addition, the composition (mainly cellulose, hemicelluloses, and pectin) of the micro-scale material can contribute to the increased rates of dissolution. Compared to cellulose and hemicelluloses, the pectin has a higher solubility for acid and alkaline pH, due to the ionization of the –COOH group (Liu, Fishman, Kost, & Hicks, 2003). Overall, the reduced particle size of the material (micro-scale) and the presence of pectin can be accounted for the higher dissolution of the sugar beet pulp. This observation clearly states that the isolated non-sucrose carbohydrate rich micro-scale tubular materials of sugar beet pulp can only be stable

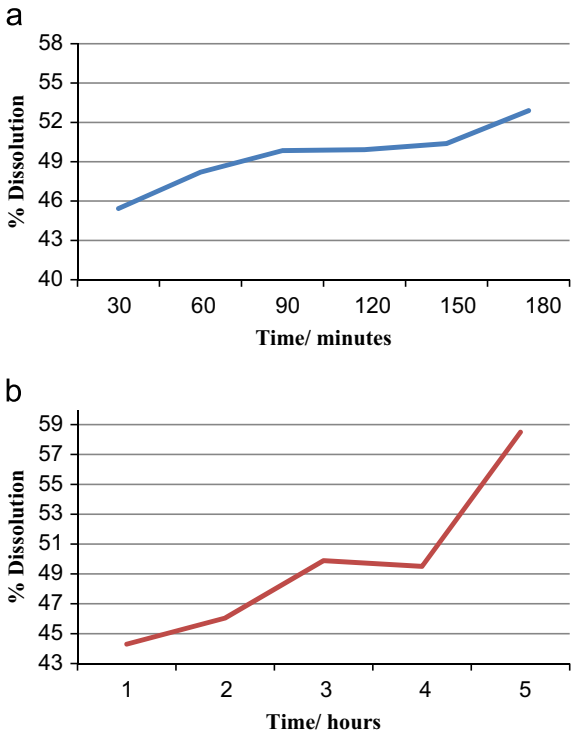

Fig. 2 – Degrees of dissolution under simulated (a) stomach (pH 1.5); and (b) intestine (pH 8.5) conditions.

in the upper gastro intestinal tract; not suitable for colon-targeted applications.

The dissolution tests were followed by imaging through scanning electron microscopy (SEM). The increased rates of dissolution were characterized by the degradation of tubular structure (Figs. 3 and 4). The degree of tubular degradation was higher at pH 8.5 compared to that of pH 1.5; the tubule integrity was lost with time and increased pH. The SEM images are in good agreement with the quantified dissolutions in Fig. 2. Thus, the dissolutions studies under simulated physiological

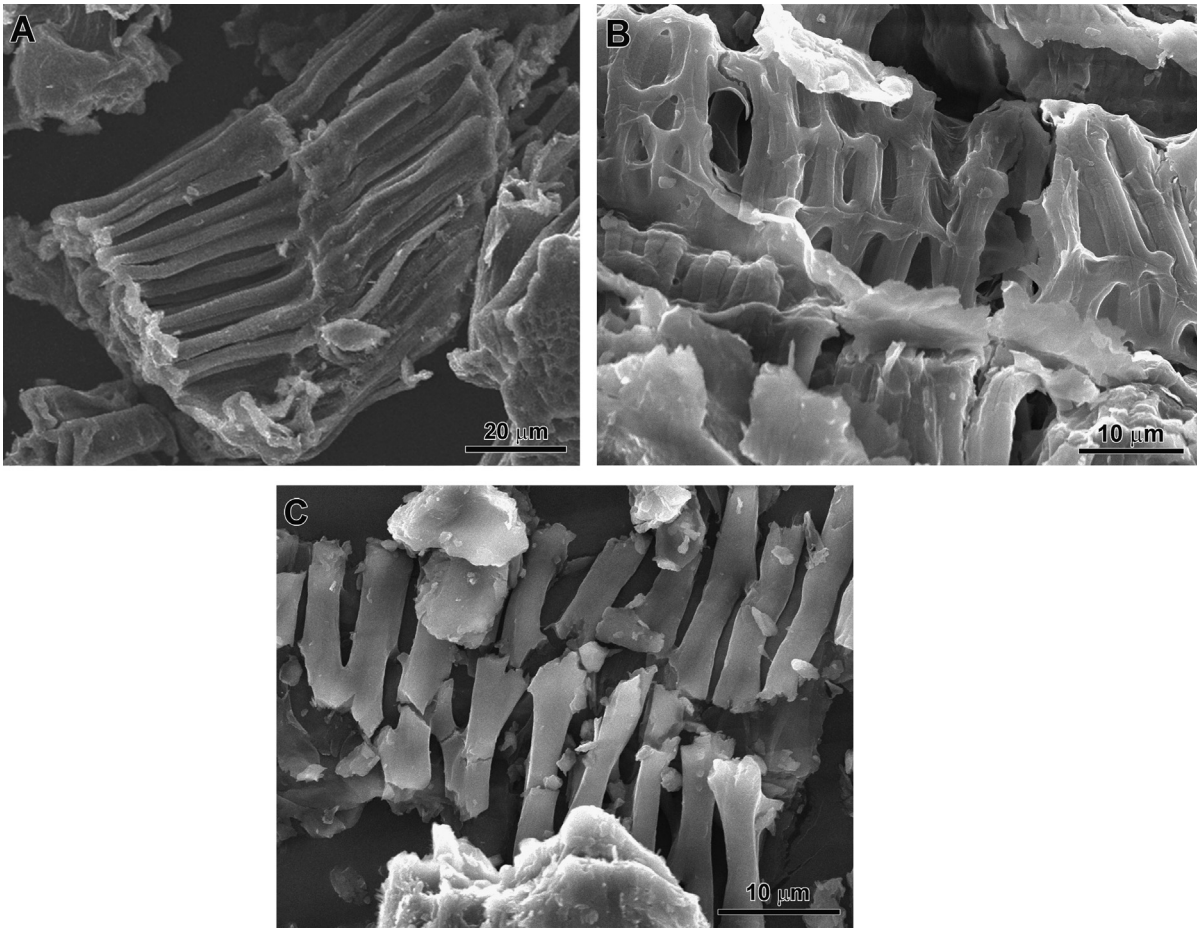

Fig. 3 – Scanning electron microscopy images under simulated stomach conditions (pH 1.5), (A) for 1 h; and (B) for 3 h.

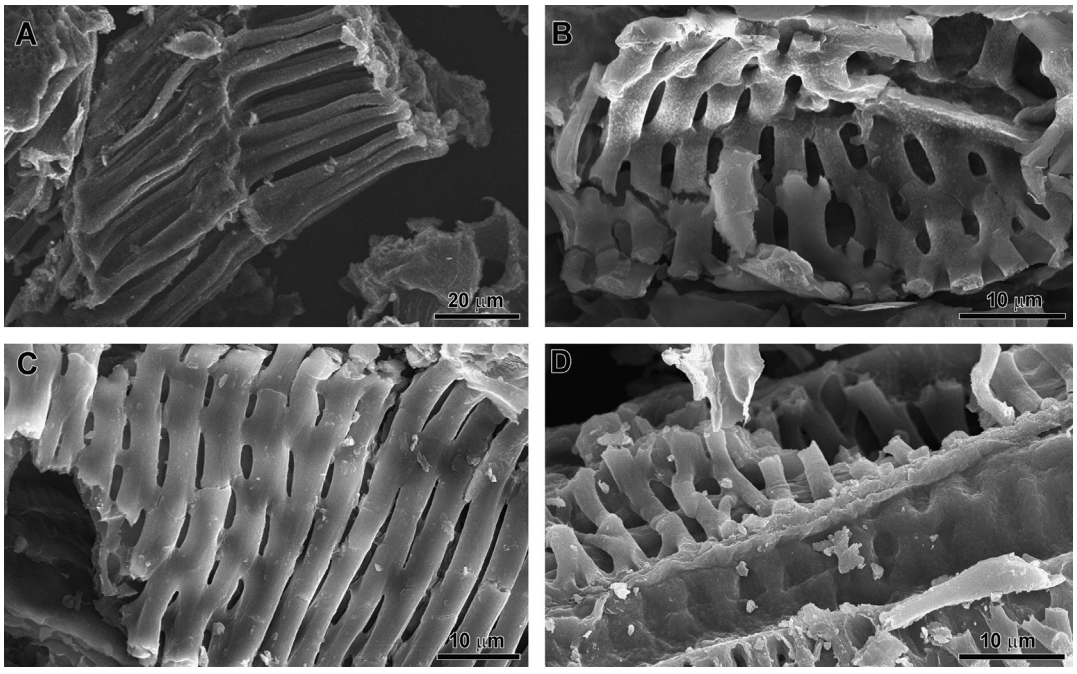

Fig. 4 – Scanning electron microscopy images under simulated intestine conditions (pH 8.5) (A) for 1 h; (B) for 3 h; and (C) for 5 h.

conditions prove that the sugar beet pulp micro-scale tubular materials can be used in micro-encapsulation for targeted delivery in the upper region of digestive system.

3.2. *Digestion tests*

Digestion test was performed to study the enzyme susceptibility of the material to pancreatic α-amylase and amyloglucosidase. The enzymatic digestion resulted in 34.1% degrees of digestion. In addition, the X-ray diffraction data showed the presence of A-type crystalline (Fig. 5) pattern (2θ=15.5 and 26) with 11.07% crystallinity. The presence of A-type pattern and lower cryatallinity represents the enzyme susceptibility of the material. Arguably, the enzyme susceptibility can most likely occur due to the presence of amorphous region; amorphous regions can be exposed to enzymatic digestion after acid hydrolysis – leading to a reduced crystallinity and formation of A-type crystal pattern (Wang, Ding, & Cheng, 2008). Furthermore, the enzyme digestion was evidenced by SEM images (Fig. 6). The enzyme digestion is characterized by a complete degradation of micro-tubular structure. The overall observation of enzyme digestion clearly shows that the micro-scale tubular structures can be degraded by pancreatic α-amylase and amyloglucosidase.

3.3. *Thermal properties*

Materials used for encapsulation in human applications should be stable under human physiological temperature. In addition, the size of the materials, macro- or micro-scale, can influence the thermal stability. To test this hypothesis, the micro-scale sugar beet pulp was subjected to DSC analysis. The thermogram obtained from DSC (Fig. 7) showed the degradation of material at 60 °C with an endothermic peak, which is in agreement with the previous study done with macro-scale sugar beet pulp (Mohamed, Biresaw, & Finkenstadt, 2007). Therefore, the DSC analysis showed that the reduced particle size did not affect the thermal stability of the material. Hence thermal stability showed that the "stacks of tubules" at micro-scale can be incorporated for human application.

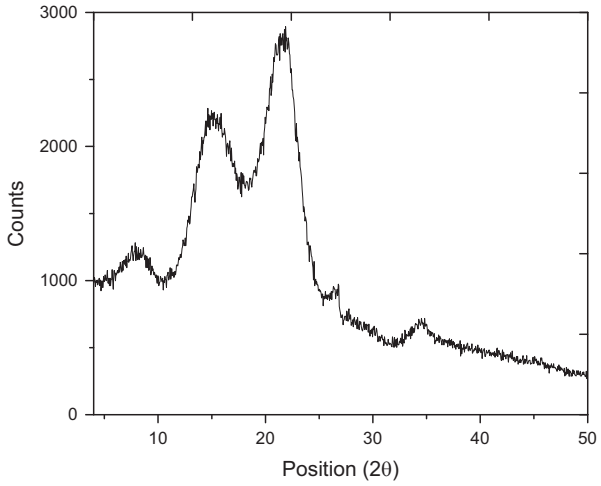

Fig. 5 – X-ray diffraction pattern of the enzymatic digestion of sugar beet pulp.

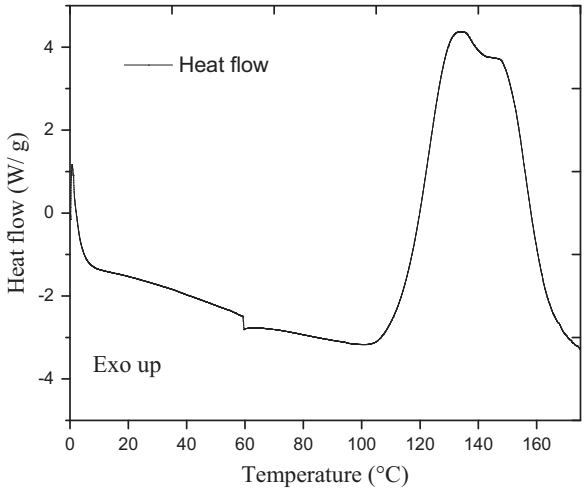

Fig. 7 – Thermal analysis (Differential scanning calorimetry) of sugar beet pulp.

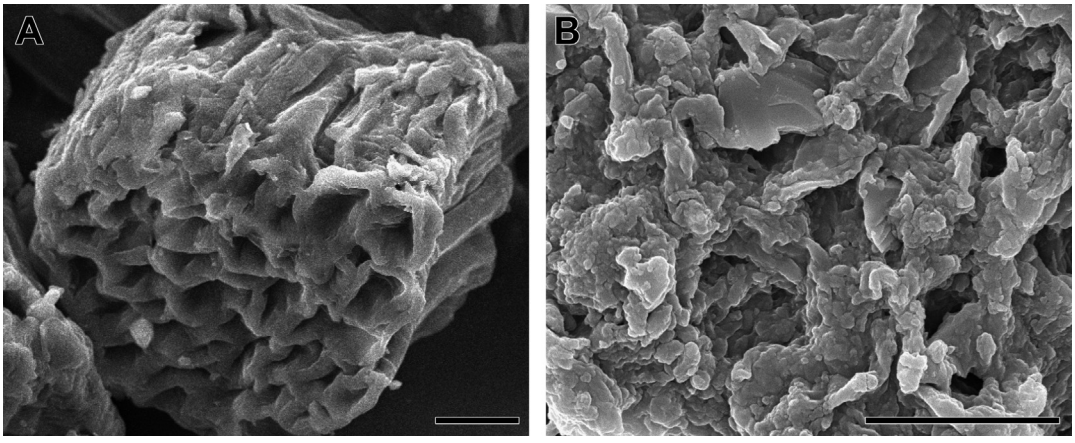

Fig. 6 – Scanning electron microscopy images of enzyme digestion of acid hydrolyzed micro scale sugar beet pulp (A) before subjecting to enzyme digestion; and (B) after subjecting to enzyme digestion.

4. Conclusion

In summary, the current study proved that the sugar beet pulp, rich in non-sucrose carbohydrates, can be used to produce micro-scale materials with unique tubular structures through acid hydrolysis. These materials were stable under simulated physiological conditions; the higher degree of dissolution suggested that the materials can be used for human application targeting the upper digestive system. In addition, the micro-scale materials were thermally stable under human physiological temperature. However, further studies will be needed to prove the efficiency of the micro-scale tubular structures of sugar beet pulp for encapsulation.

REFERENCES

Dimantov, A., Greenberg, M., Kesselman, E., & Shimoni, E. (2004). Study of high amylose corn starch as food grade enteric coating in a microcapsule model system. *Innovative Food Science & Emerging Technologies*, 5(1), 93–100, http://dx.doi.org/10.1016/j.ifset.2003.11.003.

Dimantov, A., Kesselman, E., & Shimoni, E. (2004). Surface characterization and dissolution properties of high amylose corn starch–pectin coatings. *Food Hydrocolloids*, 18(1), 29–37, http://dx.doi.org/10.1016/S0268-005X(03)00039-0.

Dinand, E., Chanzy, H., & Vignon, R. M. (1999). Suspensions of cellulose microfibrils from sugar beet pulp. *Food Hydrocolloids* 13(3), 275–283, http://dx.doi.org/10.1016/S0268-005X(98)00084-8.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fiber and fiber-rich by-products of food processing: characterisation, technological functionality and commercial applications: a review. *Food Chemistry*, 124(2), 411–421, http://dx.doi.org/10.1016/j.foodchem.2010.06.077.

Liu, L., Fishman, M. L., Kost, J., & Hicks, K. B. (2003). Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials*, 24(19), 3333–3343, http://dx.doi.org/10.1016/S0142-9612(03)00213-8.

McCleary, B. V., & Monaghan, D. A. (2002). Measurement of resistant starch. *Journal of AOAC International*, 85(3), 665–675.

Mikkonen, K. S., & Tenkanen, M. (2012). Sustainable food-packaging materials based on future biorefinery products: xylans and mannans. *Trends Food Science and Technology*, 28(2), 90–102, http://dx.doi.org/10.1016/j.tifs.2012.06.012.

Mohamed, A., Biresaw, G., & Finkenstadt, V. L. (2007). Thermal properties of poly(lactic acid) based bio-composites. In: *Proceedings of the Annual. Meeing. Adhesives Society*, 30th, 495–497.

Neethirajan, S., Tsukamoto, K., Kanahara, H., & Sugiyama, S. (2012). Ultrastructural analysis of buckwheat starch components using atomic force microscopy. *Journal of Food Science*, 77(1), N2–N7, http://dx.doi.org/10.1111/j.1750-3841.2011.02442.x.

Rashidi, L., & Khosravi-Darani, K. (2011). The applications of nanotechnology in food industry. *Critical Reviews in Food Science and Nutrition*, 51(8), 723–730, http://dx.doi.org/10.1080/10408391003785417.

Siro, I., & Plackett, D. (2010). Microfibrillated cellulose and new nanocomposite materials: a review. *Cellulose (Dordrecht, Netherlands)*, 17(3), 459–494, http://dx.doi.org/10.1007/s10570-010-9405-y.

Sozer, N., & Kokini, J. L. (2009). Nanotechnology and its applications in the food sector. *Trends in Biotechnology*, 27(2), 82–89, http://dx.doi.org/10.1016/j.tibtech.2008.10.010.

Sun, R. C., & Hughes, S. (1999). Fractional isolation and physicochemical characterization of alkali-soluble polysaccharides from sugar beet pulp. *Carbohydrate Polymers* 38(3), 273–281, http://dx.doi.org/10.1016/S0144-8617(98)00102-7.

Wang, N., Ding, E., & Cheng, R. (2008). Preparation and liquid crystalline properties of spherical cellulose nanocrystals. *Langmuir*, 24(1), 5–8, http://dx.doi.org/10.1021/la702923w.

Zhou, R., Li, Y., Yan, L., & Xie, J. (2011). Effect of edible coatings on enzymes, cell-membrane integrity, and cell-wall constituents in relation to brittleness and firmness of Huanghua pears (Pyrus pyrifolia Nakai, cv. Huanghua) during storage. *Food Chemistry*, 124(2), 569–575, http://dx.doi.org/10.1016/j.foodchem.2010.06.075.